(E) PROTEIN LOCALIZATION and tissue distribution

The human histidine protein phosphatase gene is localized at chromosome 9 (9q33 –Tel, marker sts-N90764, interval D9S159-qTEL).

Sources for the expression of cDNAs were used:

Brain, breast, CNS, colon, foreskin, germ cell, heart, kidney, liver, lung, muscle, pancreas, parathyroid, pooled, prostate, spleen, testis, thyroid, tonsil, uterus, whole embryo.

Analyzing the distribution of histidine protein phosphatasemRNA showed an increased level in normal tissues as heart, kidney, liver, pancreas, skeletal muscle and testis (Figure 9 a, b).

(F) Anti-Histidine phosphatase antibodies

Anti-Histidine phosphatase antibodies were generated against three distinct regions of the protein, namely the n-terminal, the middle and the c-terminal part of the molecule. For this puropose three peptide sequences were chosen:

peptide 1 - QIPDVDIDSD GVFKYV (16aa, SEQ. No. 9);

peptide 2 - CLGGGRISHQ SQDK (14aa, SEQ. No. 10);

peptide 3 - CTEKIKAKYP DYEV (14aa, SEQ. No. 11).

The peptides were synthesized using standard FMOC-chemistry. For immunization the peptides were injected (4 injections) each into two rabbits and four blood samples were taken. Final bleeding was taken after ca. 3 month.

The generated antibodies are usefull for detection and localization of the histidine phosphatase.

Furthermore, the different regions within the molecule can be analyzed individually. Especially the highly conserved central part of the histidine phosphatase containing the following amino acid sequence:

DCECLGGGRISHQSQD (SEQ. No. 3)

is assumed to contain the active site responsible for the proteins function in vivo. The anti-peptide antibody against this region is for inhibitory or neutralizing use.

The characteristics of the histidine protein phosphatase can be summarized as follows:

1. The amino acid sequence of human histidine protein phosphatase comprises about 124 amino acids.
2. The amino acid sequence of the histidine protein phosphatase is highly homologous in the C-terminal part but only weak homology is given for the N-terminal part.
3. The molecular weight is 13.800 +/- 100 (Figure 5).
4. The histidine protein phosphatase is N-terminal blocked by an acetylation.

Pharmaceutical Preparations

The native as well as the recombinant protein(s) may be used as a medicament which can be applied to patients directly or within pharmaceutical compositions. Thus, it is a further aspect of this invention to provide a recombinant or native protein as defined above and below applicable as a medicament and a respective pharmaceutical composition comprising said protein and a pharmaceutically acceptable diluent, carrier or excipient therefor.

The pharmaceutical compositions of the invention may contain additionally further active pharmaceutical compounds of a high diversity.

As used herein, the term "pharmaceutically acceptable carrier" means an inert, non toxic solid or liquid filler, diluent or encapsulating material, not reacting adversely with the active compound or with the patient. Suitable, preferably liquid carriers are well known in the art such as sterile water, saline, aqueous dextrose, sugar solutions, ethanol, glycols and oils, including those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil and mineral oil.

The formulations according to the invention may be administered as unit doses containing conventional non-toxic pharmaceutically acceptable carriers, diluents, adjuvants and vehicles which are typical for parenteral administration.

The term "parenteral" includes herein subcutaneous, intravenous, intra-articular and intratracheal injection and infusion techniques. Also other administrations such as oral administration and topical application are suitable. Parenteral

compositions and combinations are most preferably adminstered intravenously either in a bolus form or as a constant fusion according to known procedures. Tablets and capsules for oral administration contain conventional excipients such as binding agents, fillers, diluents, tableting agents, lubricants, disintegrants, and wetting agents. The tablets may be coated according to methods well known in the art. Oral liquid preparations may be in the form of aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or another suitable vehicle before use. Such liquid preparations may contain conventional additives like suspending agents, emulsifying agents, non-aqueous vehicles and preservatives.
Topical applications may be in the form of aqueous or oily suspensions, solutions, emulsions, jellies or preferably emulsion ointments.
Unit doses according to the invention may contain daily required amounts of the protein according to the invention, or sub-multiples thereof to make up the desired dose. The optimum therapeutically acceptable dosage and dose rate for a given patient (mammals, including humans) depends on a variety of factors, such as the activity of the specific active material employed, the age, body weight, general health, sex, diet, time and route of administration, rate of clearance, enzyme activity (units/mg protein), the object of the treatment, i. e., therapy or prophylaxis and the nature of the disease to be treated.

Therefore, in compositions and combinations in a treated patient (in vivo) a pharmaceutical effective daily dose of the protein of this invention is between about 0.01 and 100 mg/kg body weight (based on a specific activity of 100 kU/mg), preferably between 0.1 and 10 mg/kg body weight. According to the application form one single dose may contain between 0.5 and 10 mg of histidin protein phosphatase.

Short Description of the Figures

Fig. 1: Purification scheme used for the isolation of the histidine phosphatase